

CLAIMS

What is claimed is:

1. A recombinant construct comprising a promoter operably linked to a DNA sequence which, when expressed by a host produces an RNA having:

- (a) homology to at least one target mRNA expressed by the host,
- (b) two complementary RNA regions which are unrelated to any endogenous RNA in the host, and which are in proximity to (a),

wherein the expressed RNA reduces the expression of the target mRNA or any substantially similar endogenous mRNA.

2. A recombinant construct comprising a promoter operably linked to a DNA sequence which, when expressed by a host, produces an RNA having:

- (a) homology to at least one target mRNA expressed by the host,
- (b) an RNA region unrelated to any endogenous RNA in the host and located 5' to (a), and
- (c) the reverse complement of the RNA in (b) located 3' to (a),

wherein the expressed RNA reduces the expression of the target mRNA or any substantially similar endogenous mRNA.

3. A recombinant construct comprising a promoter operably linked to a DNA sequence which, when expressed by a host, produces an RNA having:

- (a) homology to at least one target mRNA expressed by the host, and
- (b) two complementary RNA regions which are unrelated to any endogenous RNA in the host, and which are located 5' to (a),

wherein the expressed RNA reduces the expression of the target mRNA or any substantially similar endogenous mRNA.

4. A recombinant construct comprising a promoter operably linked to a DNA sequence which, when expressed by a host, produces an RNA having:

- (a) homology to at least one target mRNA expressed by the host, and
- (b) two complementary RNA regions which are unrelated to any endogenous RNA in the host, and which are located 3' to (a),

wherein the expressed RNA reduces the expression of the target mRNA or any substantially similar endogenous mRNA.

5. A recombinant construct comprising a promoter operably linked to a DNA sequence which, when expressed by a host, produces an RNA having:

- (a) homology to at least one target mRNA expressed by the host, and
- (b) two complementary RNA regions which are unrelated to any endogenous RNA in the host, and which are located within (a),

wherein the expressed RNA reduces the expression of the target mRNA or any substantially similar endogenous mRNA.

6. The recombinant construct of any of Claims 1-5 wherein the RNA region or regions which are unrelated to any endogenous RNA in the host comprise a synthetic, non-naturally occurring RNA sequence.

7. The recombinant construct of any of Claims 1-5 wherein the RNA region or regions which are unrelated to any endogenous RNA in the host do not comprise plant viral RNA.

8. A method for reducing expression of a target mRNA or any substantially similar endogenous mRNA which comprises:

- (a) transforming a host with any of the recombinant constructs of Claims 1-5; and
- (b) selecting hosts which have reduced expression of the target mRNA or any substantially similar endogenous mRNA.

9. A method for reducing expression of a target mRNA or any substantially similar endogenous mRNA which comprises:

- (a) transforming a host with the recombinant construct of Claim 6; and
- (b) selecting hosts which have reduced expression of the target mRNA or any substantially similar endogenous mRNA.

10. A method for reducing expression of a target mRNA or any substantially similar endogenous mRNA which comprises:

- (a) transforming a host with the recombinant construct of Claim 7; and
- (b) selecting hosts which have reduced expression of the target mRNA or any substantially similar endogenous mRNA.

11. An RNA comprising:

- (a) homology to at least one target mRNA expressed by a host,
- (b) two complementary RNA regions which are unrelated to any endogenous RNA in the host, and which are in proximity to (a),

wherein the RNA, when introduced into the host, reduces the expression of the target mRNA or any substantially similar endogenous mRNA.

12. An RNA comprising:

- (a) homology to at least one target mRNA expressed by a host,
 - (b) an RNA region unrelated to any endogenous RNA in the host and located 5' to (a), and
 - (c) the reverse complement of the RNA in (b) located 3' to (a),
- wherein the RNA, when introduced into the host, reduces the expression of the

target mRNA or any substantially similar endogenous mRNA.

13. An RNA comprising:

- (a) homology to at least one target mRNA expressed by the host, and

(b) two complementary RNA regions which are unrelated to any endogenous RNA in the host, and which are located 5' to (a),
wherein the RNA, when introduced into the host, reduces the expression of the target mRNA or any substantially similar endogenous mRNA.

14. An RNA comprising:

- (a) homology to at least one target mRNA expressed by the host, and
- (b) two complementary RNA regions which are unrelated to any endogenous RNA in the host, and which are located 3' to (a),

wherein the RNA, when introduced into the host, reduces the expression of the target mRNA or any substantially similar endogenous mRNA.

15. An RNA comprising:

- (a) homology to at least one target mRNA expressed by the host, and
- (b) two complementary RNA regions which are unrelated to any endogenous RNA in the host, and which are located within (a),

wherein the RNA, when introduced into the host, reduces the expression of the target mRNA or any substantially similar endogenous mRNA.

16. The RNA of any of Claims 11-15 wherein the RNA region or regions which are unrelated to any endogenous RNA in the host comprise a synthetic, non-naturally occurring RNA sequence.

17. The RNA of any of Claims 11-15 wherein the RNA region or regions which are unrelated to any endogenous RNA in the host do not comprise plant viral RNA.

18. A method for reducing expression of a target mRNA or any substantially similar endogenous mRNA which comprises:

- (a) introducing into a host any of the RNA of Claims 11-15; and
- (b) selecting hosts which have reduced expression of the target mRNA or any substantially similar endogenous mRNA.

19. A method for reducing expression of a target mRNA or any substantially similar endogenous mRNA which comprises:

- (a) introducing into a host the recombinant construct of Claim 16; and
- (b) selecting hosts which have reduced expression of the target mRNA or any substantially similar endogenous mRNA.

20. A method for reducing expression of a target mRNA or any substantially similar endogenous mRNA which comprises:

- (a) introducing into a host the recombinant construct of Claim 17; and
- (b) selecting hosts which have reduced expression of the target mRNA or any substantially similar endogenous mRNA.

21. A recombinant construct comprising a promoter operably linked to a DNA sequence which, when expressed by a host produces an RNA having:

- (a) homology to at least one target mRNA expressed by the host,
- (b) two complementary RNA regions which are encoded by any nucleic acid sequence in the genome of the host provided that said sequence does not encode the target mRNA or any sequence that is substantially similar to the target mRNA and said regions are in proximity to (a),

wherein the expressed RNA reduces the expression of the target mRNA or any substantially similar endogenous mRNA.

22. A recombinant construct comprising a promoter operably linked to a DNA sequence which, when expressed by a host, produces an RNA having:

- (a) homology to at least one target mRNA expressed by the host,
- (b) an RNA region encoded by any nucleic acid sequence in the genome of the host provided that said sequence does not encode the target mRNA or any sequence that is substantially similar to the target mRNA and located 5' to (a), and
- (c) the reverse complement of the nucleic acid in (b) located 3' to (a),

wherein the expressed RNA reduces the expression of the target mRNA or any substantially similar endogenous mRNA.

23. A recombinant construct comprising a promoter operably linked to a DNA sequence which, when expressed by a host, produces an RNA having:

- (a) homology to at least one target mRNA expressed by the host, and
- (b) two complementary RNA regions which are encoded by any nucleic acid sequence in the genome of the host provided that said sequence does not encode the target mRNA or any sequence that is substantially similar to the target mRNA, and which regions are located 5' to (a),

wherein the expressed RNA reduces the expression of the target mRNA or any substantially similar endogenous mRNA.

24. A recombinant construct comprising a promoter operably linked to a DNA sequence which, when expressed by a host, produces an RNA having:

- (a) homology to at least one target mRNA expressed by the host, and
- (b) two complementary RNA regions which are encoded by any nucleic acid sequence in the genome of the host provided that said sequence does not encode the target mRNA or any sequence that is substantially similar to the target mRNA, and which regions are located 3' to (a),

wherein the expressed RNA reduces the expression of the target mRNA or any substantially similar endogenous mRNA.

25. A recombinant construct comprising a promoter operably linked to a DNA sequence which, when expressed by a host, produces an RNA having:

- (a) homology to at least one target mRNA expressed by the host, and

- (b) two complementary RNA regions which are encoded by any nucleic acid sequence in the genome of the host provided that said sequence does not encode the target mRNA or any sequence that is substantially similar to the target mRNA, and which regions are located within (a),

5 wherein the expressed RNA reduces the expression of the target mRNA or any substantially similar endogenous mRNA.

26. The recombinant constructs of Claims 21-25 wherein the nucleic acid sequence in the genome of the host is a sequence which is not expressed by the host.

10 27. The recombinant constructs of Claims 21-25 wherein the nucleic acid sequence in the genome of the host is sequence which is expressed by the host.

28. A method for reducing expression of a target mRNA or any substantially similar endogenous mRNA which comprises:

- (a) transforming a host with any of the recombinant constructs of Claims 21-25; and
- 15 (b) selecting hosts which have reduced expression of the target mRNA or any substantially similar endogenous mRNA.

29. A method for reducing expression of a target mRNA or any substantially similar endogenous mRNA which comprises:

- (a) transforming a host with the recombinant construct of Claim 26; and
- 20 (b) selecting hosts which have reduced expression of the target mRNA or any substantially similar endogenous mRNA.

30. A method for reducing expression of a target mRNA or any substantially similar endogenous mRNA which comprises:

- (a) transforming a host with the recombinant construct of Claim 27; and
- 25 (b) selecting hosts which have reduced expression of the target mRNA or any substantially similar endogenous mRNA.

31. An RNA comprising:

- (a) homology to at least one target mRNA expressed by a host,
- 30 (b) two complementary RNA regions which are encoded by any nucleic acid sequence in the genome of the host provided that said sequence does not encode the target mRNA or any sequence that is substantially similar to the target mRNA and which regions are in proximity to (a),

wherein the RNA, when introduced into the host, reduces the expression of the target mRNA or any substantially similar endogenous mRNA.

35 32. An RNA comprising:

- (a) homology to at least one target mRNA expressed by a host,
- (b) an RNA region encoded by any nucleic acid sequence in the genome of the host provided that said sequence does not encode the target mRNA or any

sequence that is substantially similar to the target mRNA and is located 5' to (a), and

(c) the reverse complement of the RNA in (b) located 3' to (a),

wherein the RNA, when introduced into the host, reduces the expression of the target mRNA or any substantially similar endogenous mRNA.

33. An RNA comprising:

(a) homology to at least one target mRNA expressed by the host, and

(b) two complementary RNA regions which are encoded by any nucleic acid sequence in the genome of the host provided that said sequence does not encode the target mRNA or any sequence that is substantially similar to the target mRNA and which regions are located 5' to (a),

wherein the RNA, when introduced into the host, reduces the expression of the target mRNA or any substantially similar endogenous mRNA.

34. An RNA comprising:

(a) homology to at least one target mRNA expressed by the host, and

(b) two complementary RNA regions which are encoded by any nucleic acid sequence in the genome of the host provided that said sequence does not encode the target mRNA or any sequence that is substantially similar to the target mRNA, and which regions are located 3' to (a),

wherein the RNA, when introduced into the host, reduces the expression of the target mRNA or any substantially similar endogenous mRNA.

35. An RNA comprising:

(a) homology to at least one target mRNA expressed by the host, and

(b) two complementary RNA regions which are encoded by any nucleic acid sequence in the genome of the host provided that said sequence does not encode the target mRNA or any sequence that is substantially similar to the target mRNA, and which are located within (a),

wherein the RNA, when introduced into the host, reduces the expression of the target mRNA or any substantially similar endogenous mRNA.

36. The RNA of any of Claims 31-35 wherein the nucleic acid sequence in the genome of the host is a sequence which is not expressed by the host.

37. The RNA of any of Claims 31-35 wherein the nucleic acid sequence in the genome of the host is a sequence which is expressed by the host.

38. A method for reducing expression of a target mRNA or any substantially similar endogenous mRNA which comprises:

(a) introducing into a host any of the RNA of Claims 31-35; and

(b) selecting hosts which have reduced expression of the target mRNA or any substantially similar endogenous mRNA.

39. A method for reducing expression of a target mRNA or any substantially similar endogenous mRNA which comprises:

- (a) introducing into a host the RNA of Claim 36; and
- (b) selecting hosts which have reduced expression of the target mRNA or any substantially similar endogenous mRNA.

40. A method for reducing expression of a target mRNA or any substantially similar endogenous mRNA which comprises:

- (a) introducing into a host the RNA of Claim 37; and
- (b) selecting hosts which have reduced expression of the target mRNA or any substantially similar endogenous mRNA.

41. A method for identifying or screening an essential plant gene which comprises:

- (a) transforming a plant cell with a recombinant construct comprising a constitutive promoter wherein said construct is capable of reducing expression of an essential plant gene with a high degree of frequency;
- (b) quantifying all transformed plant cells from step (a);
- (c) quantifying all transformed plant cells from a control which does not reduce expression of an essential plant gene; and
- (d) comparing the quantification of transformed plant cells selected from step (b) with the quantification of transformed plants cells selected from step (c) wherein the quantification of transformed plants cells selected from step (c) should substantially exceed the quantification of transformed plant cells selected from step (b).

42. A method for identifying or screening an essential plant gene which comprises:

- (a) transforming a plant cell with the recombinant construct of any of Claims 1-5 which further comprises a constitutive promoter which is capable of reducing expression of an essential plant gene with a high degree of frequency;
- (b) quantifying all transformed plant cells from step (a);
- (c) quantifying all transformed plant cells from a control which does not reduce expression of an essential plant gene; and
- (d) comparing the quantification of transformed plant cells selected from step (b) with the quantification of transformed plants cells selected from step (c) wherein the quantification of transformed plants cells selected from step (c) should substantially exceed the quantification of transformed plant cells selected from step (b).

43. A method for identifying or screening an essential plant gene which comprises:

- (a) transforming a plant cell with the recombinant construct of any of Claim 6 which further comprises a constitutive promoter which is capable of

reducing expression of an essential plant gene with a high degree of frequency;

- (b) quantifying all transformed plant cells from step (a);
- (c) quantifying all transformed plant cells from a control which does not reduce expression of an essential plant gene; and
- (d) comparing the quantification of transformed plant cells selected from step (b) with the quantification of transformed plants cells selected from step (c) wherein the quantification of transformed plants cells selected from step (c) should substantially exceed the quantification of transformed plant cells selected from step (b).

44. A method for identifying or screening an essential plant gene which comprises:

- (a) transforming a plant cell with the recombinant construct of any of Claim 7 which further comprises a constitutive promoter which is capable of reducing expression of an essential plant gene with a high degree of frequency;
- (b) quantifying all transformed plant cells from step (a);
- (c) quantifying all transformed plant cells from a control which does not reduce expression of an essential plant gene; and
- (d) comparing the quantification of transformed plant cells selected from step (b) with the quantification of transformed plants cells selected from step (c) wherein the quantification of transformed plants cells selected from step (c) should substantially exceed the quantification of transformed plant cells selected from step (b).

45. The recombinant construct of Claims 1-5 wherein the DNA sequences encoding the two complementary RNA sequences are comprised within any of the sequences set forth in SEQ ID NOs: 12, 13, or 34.